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DNA Precipitation and Hybridization (FISH)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Dextran Sulfate (50%)
Intergen, Cat. S4030
Ethanol, absolute
Formamide, deionized
Ambion, Cat. 9342
HCl, 1N
Human Cot-1 ^{TM} DNA (1mg/ml)
GIBCO BRL, Cat. 15279-011, 500 µg
Rubber Cement
Salmon Testes DNA (stock ~10 mg/ml)
SIGMA Molecular Biology, Cat. D-7657, 1ml
Sodium acetate, **3M (NaAcetate)**
20X SSC

Preparation

Master mix

Dextran sulfate, 50%	40 ml
20X SSC, pH 7	10 ml
Sterile dH_2O	50 ml

Vortex solution and place tube on a shaking platform overnight to insure proper mixing.
Aliquot and store at –20˚C

70% Formamide/2X SSC
10 ml 20 X SSC
20 ml dH_20
70 ml deionized formamide
Adjust to pH 7 with 1N HCL
Aliquot and store at –20°C.

Procedure: Precipitation

1. Add to an eppendorf tube:
   Probe DNA (600 ng -1µg DNA)
   Human Cot-1 DNA (note 1) 10 µl
   Salmon sperm DNA 1 µl

2. Add Na-Acetate, 1/10 of the total volume of probe DNA mixture (above).

3. Add 100% ethanol, i.e., 2.5 x total volume of mixture of DNA + NaAcetate.

4. Vortex, store tube at -20°C overnight or at -80°C for 30 min.

5. Centrifuge (13,000 rpm) the precipitated DNA at 4°C for 30 min.

6. Carefully pour off supernatant, and place in a speed vac for 10 min (medium heat) until pellet is dry.

7. Add 5 µl deionized formamide (pH 7.0) and incubate tube at 37°C using a thermomixer, shaking, for 30 min, and vortex several times during the incubation.

8. Add 5 µl Master Mix, vortex, centrifuge briefly.

9. Denature probe DNA at 80°C for 5 min, centrifuge briefly.

10. Pre-anneal probe at 37°C for 1 hr.

Procedure: Slide Denaturation and Hybridization

1. Apply 120 µl 70% deionized formamide/2X SSC to a 24 mm x 60 mm coverslip. Touch the slide to the coverslip.

2. Denature slide at 80°C on a hot plate for 1.5 min (see note 1).

3. Quickly and carefully remove the coverslip and immediately place the slide in ice cold 70% ethanol, followed by 90% ethanol and 100% ethanol (for 3 min each).

4. Allow slides to air dry.

5 Add pre-annealed probe DNA to the denatured slide and cover with an 18 mm^2 coverslip, carefully remove any bubbles with forceps, and completely seal the edges of the coverslip with rubber cement.

6 Hybridize at 37°C in hybridization chamber (use a light tight chamber if the probe is directly labeled) for 48 hr.

Notes

1. cDNA probes do not require Cot-1 DNA.

2. The denaturation time depends on the age of the slide. For slides older then 30 days a denaturation time of 2 min is recommended.